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Trimerization and crystallization of reconstituted light-harvesting chlorophyll *a/b* complex

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The major light-harvesting complex (LHCII) of photosystem II, the most abundant chlorophyll-containing complex in higher plants, is organized in trimers. In this paper we show that the trimerization of LHCII occurs spontaneously and is dependent on the presence of lipids. LHCII monomers were reconstituted from the purified apoprotein (LHCP), overexpressed in *Escherichia coli*, and pigments, purified from chloroplast membranes. These synthetic LHCII monomers trimerize *in vitro* in the presence of a lipid fraction isolated from pea thylakoids. The reconstituted LHCII trimers are very similar to native LHCII trimers in that they are stable in the presence of mild detergents and can be isolated by partially denaturing gel electrophoresis or by centrifugation in sucrose density gradients. Moreover, both native and reconstituted LHCII trimers exhibit signals in circular dichroism in the visible range that are not seen in native or reconstituted LHCII monomers, indicating that trimer formation either establishes additional pigment–pigment interactions or alters pre-existing interactions. Reconstituted LHCII trimers readily form two-dimensional crystals that appear to be identical to crystals of the native complex.

Key words: 2D crystals/antenna/LHCII/oligomerization/thylakoid lipids

Introduction

Light-harvesting complexes greatly enhance the efficiency of the photosynthetic apparatus in higher plants. They increase by several hundred-fold the probability that a light quantum will be absorbed and reach the photosynthetic reaction centre (Sauer, 1986). The most abundant of these complexes is the major chlorophyll *a/b*-containing light-harvesting complex of photosystem II (LHCII) which comprises about one-third of the total amount of thylakoid protein. LHCII contains an apoprotein (LHCP) of 25–28 kDa which non-covalently binds at least 12 chlorophylls (Kühlbrandt *et al.*, 1994), two luteins and one other xanthophyll (Bassi *et al.*, 1993; Juhler *et al.*, 1993) as well as lipids (Rémy *et al.*, 1982; Nussberger *et al.*, 1993). Three types of LHCP, called Lhb1, Lhb2 and Lhb3 (Jansson *et al.*, 1992), have been found in all plants examined so far, having an amino acid sequence similarity of ~90% or higher. The functional significance of these various types of LHCP is not yet clear.

In fully green plants, LHCII appears to be organized in trimers which are stable enough to be isolated from detergent-solubilized thylakoid membranes (Burke *et al.*, 1978). Little is known so far about how these trimeric complexes are stabilized or about the significance and regulation of the trimerization step in the biogenesis of the photosynthetic antenna. Lipids, particularly phosphatidyl glycerol lipids, appear to be involved in the stabilization of trimers (Rémy *et al.*, 1984) by interacting, directly or indirectly, with the N-terminal domain of LHCP (Nussberger *et al.*, 1993). Phosphatidylglycerol appears to be an integral part of the monomeric complex (Nussberger *et al.*, 1993).

LHCP–pigment complexes can be reconstituted *in vitro* in the presence of detergents. The resulting complexes closely resemble monomeric complexes of native LHCII regarding their stability and electrophoretic behaviour, spectroscopic properties and pigment composition (Plumley and Schmidt, 1987; Paulsen *et al.*, 1990). By analysing the pigment binding properties of mutated versions of LHCP it has been possible to distinguish between protein domains that are important for the formation or stabilization of LHCP–pigment complexes and others that are not (Cammarata *et al.*, 1992; Paulsen and Hobe, 1992). Moreover, monitoring the protein structure during the reconstitution assay by circular dichroism spectroscopy revealed that pigment binding *in vitro* involves a pigment-induced conformational change of the protein (Paulsen *et al.*, 1993).

High-resolution structure analysis by electron crystallography of two-dimensional crystals of native LHCII at 3.4 Å resolution (Kühlbrandt *et al.*, 1994) has revealed three membrane-spanning α-helices and one amphipathic helix on the membrane surface in the polypeptide. Twelve chlorophyll tetrapyrrole rings were resolved and assigned to chlorophyll *a* or *b* based on their proximity to the two lutein molecules which were found in the centre of the complex. Eight amino acid side chains acting as chlorophyll ligands have been identified. For future studies of the structure and function of LHCII it will be important to modify the complex by site-directed mutagenesis.

So far, only the trimeric form of the complex has been crystallized. Trimerization of the material reconstituted *in vitro* is therefore an important prerequisite for structure studies at high resolution. In order to study the trimerization of LHCII, we set out to establish an *in vitro* procedure for oligomerizing reconstituted LHCP–pigment complexes. The crystallization of reconstituted LHCII would open up the possibility of correlating specific mutations in the protein with alterations in the overall structure of the pigment-containing complex, thus greatly

facilitating the assessment of the protein structure in terms of its pigment binding function. Moreover, crystals of reconstituted LHCII containing a homogeneous population of LHCP are expected to yield data at higher structural resolution than crystals of native LHCII which always contain a mixture of the different LHCP molecules of various types.

In this paper, we show that reconstituted LHCII can be trimerized in the presence of lipids, and that these trimers indeed form crystals of identical appearance to crystals from native LHCII trimers.

Results

Trimerization of reconstituted LHCII is dependent on the presence of lipids

LHCP–pigment complexes can be reconstituted *in vitro* from the apoprotein and pigments in the presence of dodecyl sulfate as a detergent (Plumley and Schmidt, 1987; Paulsen *et al.*, 1990). These complexes closely resemble monomeric LHCII regarding their biochemical and spectroscopic properties. The trimeric form of LHCII as isolated from plant thylakoids is unstable in the presence of the high concentrations of dodecyl sulfate used for reconstitution. Therefore, oligomerization of the reconstituted complexes under reconstitution conditions is not to be expected.

We tried to stimulate trimerization of reconstituted LHCII by exchanging dodecyl sulfate after completion of the reconstitution procedure with non-ionic detergents such as octyl glucoside or dodecyl maltoside in which trimeric LHCII is stable. In most experiments, no oligomerization of the complexes was observed under these conditions (Figure 1C). In some experiments we obtained spurious amounts of oligomeric LHCII which may be correlated with a contamination by lipids of the pigment preparations used for reconstitution.

Significant amounts of reconstituted LHCII trimers were obtained only when we isolated the LHCP–pigment complexes after reconstitution and subsequently suspended them in lipid vesicles. The lipids were isolated from green pea thylakoids and depleted of monogalactosyldiacyl glyceride (MGDG) in order to facilitate vesicle formation. Solubilization of these vesicles in non-ionic detergents yielded ~10% of trimeric LHCII which could be separated from the monomeric form by centrifugation in a sucrose density gradient (Figure 1B). Yields of up to 40% LHCII trimers have been achieved under optimal conditions (results not shown).

Reconstituted LHCII trimers have the same biochemical characteristics as native LHCII trimers

In Figure 1 the sedimentation in a sucrose density gradient of the oligomerization product of reconstituted LHCII (B) is compared with that of native LHCII (A). The two samples show the same pattern of bands, assigned, in the order of decreasing velocity of sedimentation, to LHCII trimers, monomers, and micelles containing unbound pigment. Native LHCII trimers tend to aggregate when insufficient amounts of the solubilizing detergent are

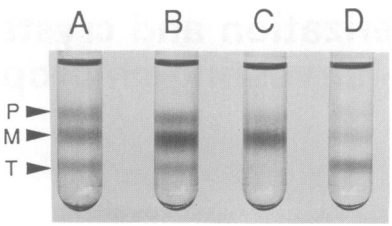

Fig. 1. Separation of LHCII monomers and trimers by sucrose density centrifugation. The samples loaded on analytical sucrose density gradients (see Materials and methods) were: (**A**) native LHCII isolated from pea and partially denatured in 1% OG by a short heat treatment (2 min at 65 °C); (**B**) reconstituted LHCII monomers trimerized in the presence of lipids; (**C**) reconstituted LHCII monomers treated in the same way as the ones in B except that no lipids were added; (**D**) reconstituted LHCII trimers isolated by preparative sucrose density centrifugation. P: unbound pigment; M: monomers; T: trimers.

present, resulting in a band sedimenting more quickly than solubilized LHCII trimers (Bassi *et al.*, 1991). However, the sample of native LHCII (A) was prepared by partial dissociation of isolated LHCII trimers into monomers by incubation for 2 min at 65°C in 1% octyl glucoside. Since the more rapidly sedimenting band does not shift to lower mobility upon this treatment, we conclude that this band in the sucrose gradients with both native and reconstituted LHCII (A and B, respectively) contains trimers rather than higher aggregation states of LHCII.

The sedimentation velocity of a purified sample of reconstituted trimeric LHCII is preserved after precipitation and resolubilization in 0.1% LM (Figure 1D). The appearance of minor amounts of unbound pigments and monomeric LHCII in the sample is due to partial disintegration upon this treatment. The fact that native trimeric LHCII partially disintegrates at a comparable rate when treated in the same way (not shown) suggests a similar stability of reconstituted and native trimers.

In order to confirm further the nature of the rapidly sedimenting component in the preparation of oligomerized reconstituted LHCII as the trimeric form, bands containing protein were isolated from the sucrose gradients and subjected to partially denaturing gel electrophoresis (Figure 2). The middle and lower bands of the sucrose gradient containing native LHCII (lanes A/M and A/T, respectively) and of the gradient containing reconstituted LHCII (lanes B/M and B/T) showed the electrophoretic mobility expected for LHCII monomers and trimers, respectively. Thus, in both sucrose density gradients and partially denaturing 'green' gel electrophoresis, the oligomerized reconstituted LHCII behaves in the same way as native LHCII trimers.

We measured the pigment stoichiometry in the reconstituted trimeric LHCII and found no significant change as compared with the monomeric complexes within our limits of accuracy (~1 chlorophyll molecule/protein monomer, data not shown). We did not add any additional pigment to the trimerization reaction containing reconstituted LHCII and lipids, and thus have no indication of trimeric LHCII binding more pigments than the ones bound to the individual monomers. However, during the trimerization procedure we always observed the appearance of some free pigment due to the breakdown of LHCII. Therefore,

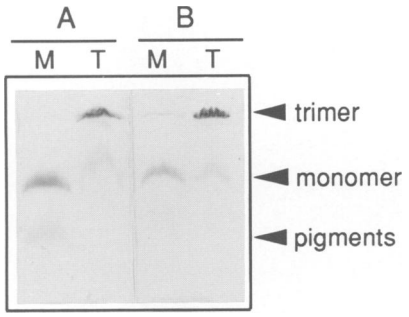

Fig. 2. Partially denaturing gel electrophoresis of LHCII monomers and trimers separated on sucrose density gradients. Sucrose density gradients A (native sample) and B (reconstituted sample) from Figure 1 were fractionated from the top and the separated bands containing protein were loaded directly on partially denaturing 'green' polyacrylamide gels. Lanes M and T correspond to the monomeric (M) and trimeric (T) bands in the gradients (Figure 1). The bands seen in this figure represent green bands on the non-stained gel.

we cannot exclude the binding of some additional pigments during the trimerization of reconstituted LHCII.

Trimerization of reconstituted LHCII generates the same CD signals as found in native LHCII trimers

Another line of evidence for the formation of structurally intact trimers from reconstituted LHCII comes from the circular dichroism (CD) in the visible range, a monitor for close pigment–pigment interactions. Figure 3 shows CD spectra recorded from the monomeric (A and B) and trimeric (D) forms of native LHCII prepared from thylakoids, isolated on a sucrose density gradient (A and D) or on a partially denaturing gel (B). CD spectra of *in vitro* reconstituted monomers (C) and trimers (E) are very similar to those of the corresponding native complexes. Three distinct differences can be seen between the spectra from monomeric (A, B and C) and trimeric (D and E) LHCII. (i) In the Q_y range there is a rather weak but significant shoulder at 648 nm which only appears in the trimer spectra. This signal was first described by Gülen *et al.* (1986) and is more pronounced in CD spectra recorded at 77 K (Hemelrijk *et al.*, 1992). (ii) A rather prominent negative signal at 478 nm in CD spectra of LHCII trimers is missing both in spectra of native LHCII monomers separated on a 'green' gel (B) and in spectra of reconstituted monomers isolated from sucrose density gradients (C). This signal is largely reduced in spectra of native monomers isolated on a sucrose density gradient (A). (iii) A third visible although less significant difference appears as a rather broad positive signal at 412 nm in the trimer spectra which is absent in the monomer spectra.

Reconstituted monomeric LHCII (C) exhibits the same CD signals as native LHCII monomers isolated on a partially denaturing gel (B) but lacks the signal at 478 nm which is visible in the spectra of native LHCII monomers isolated on a sucrose density gradient (A). We cannot interpret this difference at this point. However, the trimer spectra of reconstituted LHCII show all the additional signals at 648, 478 and 412 nm that distinguish the spectra of native LHCII trimers from those of the monomers. We cannot assign these signals to specific pigment interactions at the present state of our knowledge.

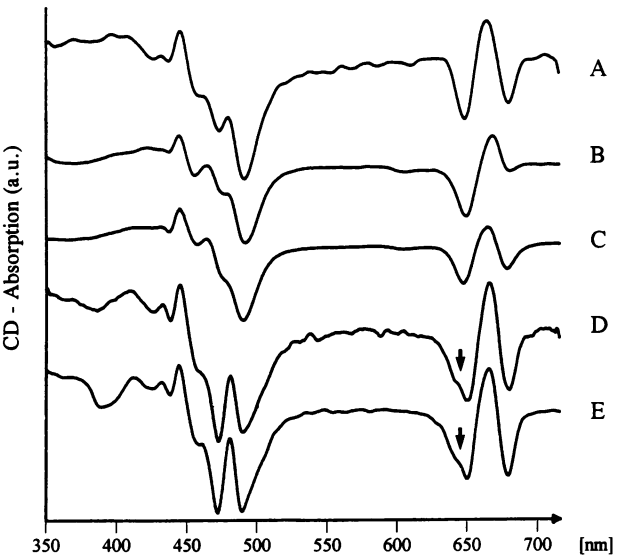

Fig. 3. CD spectra of native and reconstituted LHCII monomers and trimers. CD spectra were measured either with LHCII monomer or trimer fractions isolated on sucrose density gradients as in Figure 1 (**A**, **C**, **D** and **E**) or gel slices of partially denaturing polyacrylamide gels containing green bands as in Figure 2 (**B**). The samples contained native LHCII monomers (A and B), reconstituted monomers (C), native trimers (D), and reconstituted trimers (E). The arrows indicate the trimer specific shoulder at 648 nm.

However, it is very likely that these signals arise from pigment interactions which are present in the trimer but absent in the monomer. These are likely to include interactions between chlorophylls a_4 and a_5, a_4 and b_5, a_3 and a_7 in adjacent monomers which have Mg–Mg distances of 16–17 Å in the high resolution structure (Kühlbrandt *et al.*, 1994).

Two-dimensional crystallization of reconstituted LHCII

Two-dimensional (2D) crystals of reconstituted LHCII were grown under conditions identical to those developed for the native complex (Kühlbrandt, 1984; Wang and Kühlbrandt, 1991), except that it was necessary to supply extra digalactosyldiacyl glyceride (DGDG), as for the crystallization of partially delipidated native LHCII (Nussberger *et al.*, 1993). The 2D crystals were examined in negative stain by electron microscopy and found to be indistinguishable from those of the native control (Figure 4) in terms of lattice dimension and symmetry (a = 130 Å, layer group p321). Differences in appearance are fully accounted for by stain penetration which can vary substantially between specimens, and by the exact electron optical focus used for recording the image. Image analysis of the best of a series of negatively stained crystals yielded a projection map (Figure 5B) which indicated that the structure of the reconstituted LHCII trimer is at least very similar to that of the native control (Figure 5A) at low resolution, as is shown by the position of peaks and troughs in the projection map. Differences between the two maps are most likely due to the fact that the control (Figure 5A) represents an average over many highly selected images whereas data used to generate Figure 5B

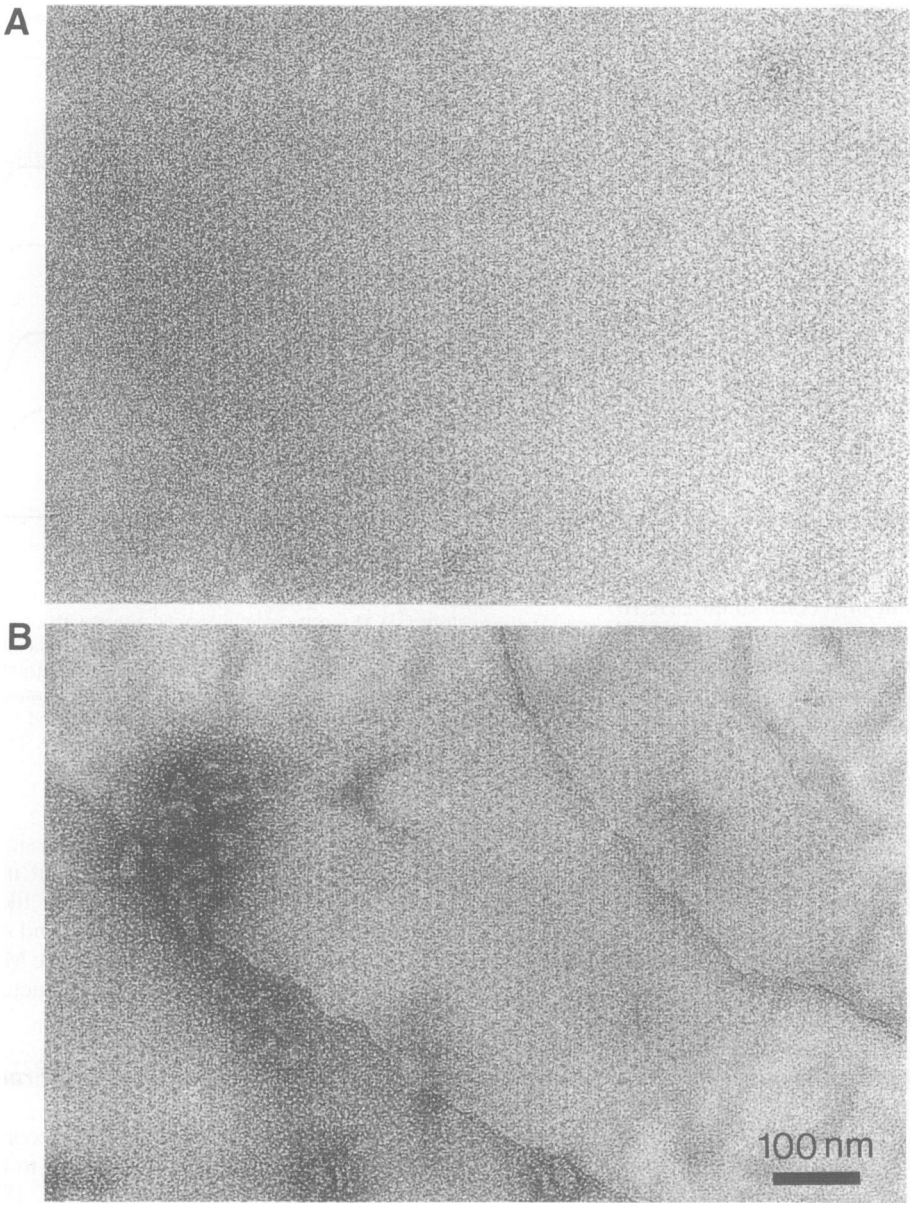

Fig. 4. Two-dimensional crystals of LHCII contrasted with a negative stain, uranyl acetate. Crystals were grown from (**A**) native LHCII trimers isolated from pea chloroplasts (control); (**B**) reconstituted trimers. Both crystals show the characteristic honeycomb lattice and the same unit cell (a = 130 Å). Steps in panel B are probably due to a lamellar phase of the lipids used for reconstitution.

were derived from a single image and are therefore noisier and less complete.

Discussion

Structure of LHCII trimers

Reconstituted LHCII monomers do not associate in detergent solution. They do, however, form trimeric complexes after being inserted into lipid vesicles. The oligomerization of LHCII monomers in liposomes has been described previously for LHCII preparations from tobacco thylakoids by preparative SDS–gel electrophoresis (Rémy *et al.*, 1982, 1984). There are two possibilities for how lipids could stimulate trimer formation. Either lipids are a constitutive component of trimeric LHCII or the trimeric complexes need to be stabilized by insertion into a lipid bilayer. The first explanation is favoured by several lines of evidence. (i) Reconstituted or native LHCII trimers are stable after solubilization with a mild detergent; the lipid environment is therefore not needed for structural stability. (ii) Lipids, particularly phosphatidyl glycerol, remain tightly bound to detergent-solubilized LHCII trimers (Rémy *et al.*, 1982, 1984) and monomers (Nussberger *et al.*, 1993). (iii) LHCII trimers in or isolated from thylakoids irreversibly dissociate into monomers when they are treated with phospholipase (Rémy *et al.*, 1982; Nussberger *et al.*, 1993).

There is good evidence that LHCII trimers are stabilized partly by interaction between the monomers at the N-terminal regions of their apoproteins (Kühlbrandt and Wang, 1991; Nussberger *et al.*, 1993). The appearance of new CD signals in the visible range upon trimer formation of LHCII suggests that chlorophyll–chlorophyll contacts are also involved in the formation of the oligomer although

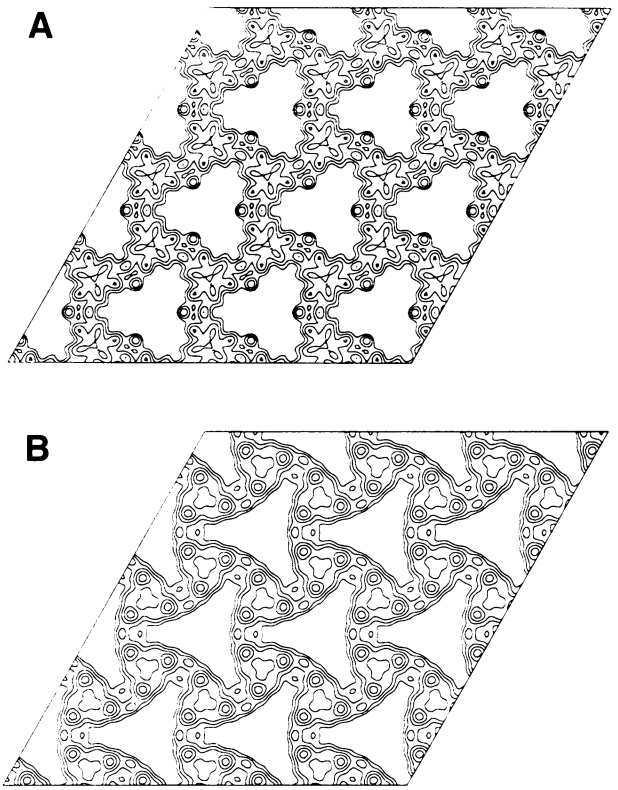

Fig. 5. (**A**) Average projection map of pea LHCII, derived from the three-dimensional data set of negatively stained crystals (Kühlbrandt, 1984) at the same resolution as in B. (**B**) Projection map of LHCII derived from the crystal of reconstituted LHCII shown in Figure 4B. The crystal symmetry is the same (p321) in both cases. The position of peaks in the trimer indicates that the structure is very similar.

it is also possible that trimerization establishes or alters some pigment–pigment interactions within the monomers. We have no direct evidence that trimerization generates a new chlorophyll binding site but we cannot exclude the binding of some additional pigment. Regardless of these considerations, the apparently trimer-specific CD signals at 648 and 478 nm are useful as a monitor for LHCII oligomerization in different environments without the necessity of separating trimers from monomers.

Lipid-dependent oligomer formation of reconstituted membrane protein complexes

Functional LHCII monomers can be reconstituted *in vitro* in the absence of lipids (Plumley and Schmidt, 1987; Paulsen *et al.*, 1990), whereas lipids are required for LHCII trimer formation. Lipid-dependent oligomerization has been reported for other reconstituted membrane protein complexes. The first membrane protein to be successfully reconstituted was bacteriorhodopsin which apparently forms spontaneously when lipids, cholate and retinal are added to the SDS-solubilized apoprotein (Huang *et al.*, 1981). For the reconstitution of monomeric bacteriorhodopsin, lipids are dispensable (London and Khorana, 1982). When bacteriorhodopsin is reconstituted in a lipid-detergent mixture and the detergent is subsequently removed by dialysis, the protein complex forms crystalline lattices (London and Khorana, 1982; Popot *et al.*, 1986). which have been used to localize some of the helices within the protein by neutron scattering (Trewhella *et al.*, 1986).

Lipid-dependent assembly into oligomers has also been shown for reconstituted porin (Eisele and Rosenbusch, 1990). On the other hand, the α and β subunits of the light-harvesting complex from *Rhodospirillum* can be reconstituted with bacteriochlorophyll *a* and assembled into the B873 complex in lipid-free detergent solution. However, the CD spectrum of this *in vitro*-assembled complex becomes similar to the CD signal of the native complex only upon the addition of lipids during the reconstitution procedure, indicating that reconstituted B873, though stable in detergent solution, requires lipids in order to adopt a more native-like structure (Parkes-Loach *et al.*, 1988).

To our knowledge, in neither of these studies was a recombinant, bacterially expressed protein used or the structure of assembled oligomers examined by electron microscopy of single 2D crystals. We are presently investigating the effect of specific kinds of lipids on oligomer formation of reconstituted LHCII.

Biogenesis of LHCII trimers

Little is known about the trimerization step during LHCII biogenesis. In greening pea leaves, LHCII monomers are accumulated 12–24 h prior to the emergence of trimeric complexes and then gradually disappear as the level of trimeric complexes rises (Jaing *et al.*, 1992). When LHCII monomers are pulse-labelled during the first hours of greening, the label is observed to shift subsequently into the LHCII trimer fraction (B.A.W.Dreyfuss and J.P.Thornber, personal communication). These data indicate that LHCII monomers are assembled first and then incorporated into an oligomeric structure.

Our results show that monomeric LHCII has an intrinsic capacity for forming trimeric complexes in the presence of lipids, excluding other components as constitutive parts of LHCII trimers. Furthermore, our data prove that the heterogeneity in LHCP observed *in vivo* is not a prerequisite for the formation of trimeric complexes. It is unknown as yet whether Lhb1- and Lhb2-type LHCII monomers form homo-trimers or hetero-trimers of a fixed or variable stoichiometry (Spangfort and Andersson, 1989; Allen and Staehelin, 1992) and whether Lhb3 is present in LHCII oligomers at all (Peter and Thornber, 1991). Our reconstituted LHCII trimers correspond to an Lhb1 homo-trimer. We are currently investigating the trimerization of mutagenized LHCP derivatives including such with typical sequence features of Lhb2 and Lhb3 in order to compare the stabilities of the various homo- and hetero-trimers.

Crystallization of reconstituted LHCII

Reconstituted LHCII forms 2D crystals under conditions identical to those needed for crystallization of the native complex, and the resulting crystals are indistinguishable. This means that the molecular structure of the reconstituted material must be very close to that of the native complex. The interaction between reconstituted monomers in the trimer must likewise be very similar or identical. The structure of native, trimeric LHCII has recently been determined at near-atomic resolution by high-resolution electron microscopy of 2D crystals (Kühlbrandt *et al.*, 1994). High-resolution electron diffraction patterns of

unstained 2D crystals of reconstituted LHCII have been obtained (D.Madden, S.Hobe, H.Paulsen and W.Kühlbrandt, unpublished results) and will be used for a detailed comparison of the structure of the reconstituted complex with that of the native LHCII. We hope that these studies will make it possible to examine effects of amino acid substitutions on the structure and function of LHCII in particular and on membrane protein oligomerization in general.

Materials and methods

Preparation of monomeric complexes

Purification of overexpressed LHCP and isolation of plant pigments was as described elsewhere (Paulsen *et al.*, 1990). For the present study a LHCP clone derived from the AB80 gene (Cashmore, 1984) from pea (*Pisum sativum*) was used. The N-terminal sequence (MRATT...) is slightly altered relative to the mature wild-type (MRKSATT...) in that amino acids 3 and 4 are missing due to the cloning strategy.

In contrast to former studies on the reconstitution of LHCII monomers which took place on an analytical scale (Paulsen and Hobe, 1992; Paulsen and Kuttkat, 1993) the preparation of trimeric LHCII in amounts sufficient for crystallization required a scaling up of the reconstitution procedure by more than three orders of magnitude. A similar procedure for a large-scale preparation of reconstituted LHCII suitable for NMR measurements will be published elsewhere (S.Prytulla, S.Hobe, H.Paulsen and H.Oschkinat, submitted).

Standard reconstitution assays (20–50 ml) contained 0.4 mg/ml LHCP, 1 mg/ml chlorophyll (chlorophyll *a*:*b* = 1) and xanthophylls (0.15 mg/ml) in reconstitution buffer [2% lithium dodecyl sulfate (LDS), 100 mM Tris–HCl pH 9.0, 12.5% sucrose (w/v), 5 mM ε-aminocaproic acid, 1 mM benzamidine]. LHCP was first solubilized in reconstitution buffer for 5 min at 100°C. Dithiothreitol was added to a final concentration of 1 mM after the sample had cooled down to room temperature. Pigments were dissolved in ethanol [7% (v/v) of the LHCP solution], injected into the LHCP solution in a bath-sonifier and sonified for another 5 min. After at least three freeze–thaw cycles (freezing at –20°C, thawing at room temperature) complexes were thawed on ice and octyl glucoside was added to a final concentration of 1%. After stirring for 10 min at 0°C, 2 M KCl was added to a final concentration of 200 mM. Precipitated potassium dodecyl sulfate was removed from the solubilized complexes by centrifugation (5 min, 8000 *g*) after another 10 min of stirring at 0°C. The sample was then desalted on a Sephadex G-25 gel filtration column (Pharmacia, Freiburg, Germany) of 200 ml volume at 5.5 cm diameter, equilibrated with column buffer (0.7% Triton X-100, 3 mM sodium phosphate, pH 7.4). After adding NG to a final concentration of 0.7%, the material was applied to an anion-exchange column (Fractogel TSK DEAE-650 (S), Merck, Darmstadt, Germany; column volume 30 ml with 2 cm diameter), equilibrated with column buffer. Unbound pigments were partially eluted with ~1.5 column volumes of washing buffer [0.5% nonyl glucoside (NG), 3 mM sodium phosphate, pH 7.4]. For the elution of bound complexes, the green zone of column material (~5 ml) was suspended in 0.5 volumes of elution buffer (500 mM NaCl, 1.0% NG, 3 mM sodium phosphate pH 7.4), the Fractogel was pelleted (5 min, 8000 *g*) and the supernatant removed. This batch elution procedure was repeated until the supernatant was virtually colourless (four or five times). The combined supernatants were diluted into 20 ml of ice-cold 10 mM Tris–HCl (pH 7.0). Subsequent desalting and removal of NG was achieved by a combination of dialysis and ultrafiltration. The sample was first dialysed overnight in 5 litres of 10 mM Tris–HCl (pH 7.0), 1 mM dithiothreitol and then diluted 10-fold in buffer T [5 mM tricine (pH 7.5), 50 mM sucrose, 0.5 mM EDTA]. This diluted sample was concentrated in an ultrafiltration cell (Amicon, mol. wt cut-off: 30 000 Da) to a final volume of ~2 ml. During this procedure most of the material should precipitate. The suspension of monomeric LHC was kept at –20°C. Chlorophyll contents of several preparations were 5–10 mg/ml with unbound pigments in the range 30–50% of total chlorophyll.

Isolation of lipids

Chloroplasts were isolated as described in Mullet *et al.* (1986) except that the enrichment of intact chloroplasts by a Percoll gradient centrifugation was omitted. Chloroplasts were lysed in 10 mM HEPES–KOH (pH 8.0). Subsequent purification of thylakoid lipids was achieved by column chromatography on silicic acid (Sigma, Munich, Germany) following a method described by Sprague and Staehelin (1987). All organic solvents were saturated with N_2 and contained butylated hydroxytoluene (1 μg/ml). Chromatography and all subsequent steps were performed at 4°C. The bulk of pigments was first eluted with chloroform. Bound lipids were depleted of MGDG by elution with chloroform/acetone (1:1) prior to the elution of remaining lipids (chloroform/methanol, 1:1). The absence of MGDG was verified by TLC (Alugram Sil G, Macherey & Nagel, Düren, Germany; solvent system: $CHCl_3/CH_3OH/H_2O$ = 65:25:4). MGDG was excluded because of its tendency to form a hexagonal phase in aqueous suspensions (Shipley *et al.*, 1973) thereby preventing the formation of a homogenous population of lipid vesicles of known concentration. Eluted fractions which did not contain MGDG were pooled, evaporated to dryness and resuspended in buffer T at a concentration of 10 mg/ml by sonification. This stock suspension of lipid vesicles was saturated with N_2 and kept at –196°C until use.

Trimerization

The insertion of LHC into lipid vesicles is based on a procedure described by Ryrie *et al.* (1980) with some modifications. Standard assays contained 1 mg/ml lipid and 1 mg/ml LHCP complexed with pigments in buffer T. Both lipid vesicles and monomeric LHCII were thawed at 0°C and sonified for 5 min and 1 min, respectively. After mixing the two components, the sample was sonified for 1 min, frozen at –196°C and thawed in a water bath (22°C). Immediately after thawing, the sample was loaded on a 7% solution of Ficoll in buffer T and pelleted (19 h, 150 000 *g*). The supernatant was removed and the pellet was solubilized in 0.1% dodecyl-β,D-maltoside (LM) for at least 30 min at 0°C. After precipitating non-solubilized material (2 min at 12 000 *g* and 4°C), the sample was loaded on a sucrose density gradient (10–45% sucrose, 0.1% LM for preparative assays, 12.5–25% sucrose, 0.1% LM for analytical gradients). The sucrose gradient was centrifuged at 300 000 *g* for 18 h at 4°C. The resulting green bands (unbound pigment, monomeric LHCII, trimeric LHCII) were removed separately. For subsequent crystallization, LM was removed from the fraction containing LHCII-trimers with Biobeads (Bio-Rad, Munich, Germany) and the complex was precipitated with 300 mM KCl.

Circular dichroic spectra

CD spectra were taken at 4°C using a Dichrograph CD7 (Jobin-Yvon, Longjumeaux, France). Blank spectra for bands removed from sucrose gradients contained corresponding amounts of sucrose and LM whereas blank spectra for material in the gel were taken from colorless gel slices.

Partially denaturing gel electrophoresis was carried out as described earlier (Paulsen and Hobe, 1992)

Two-dimensional crystallization, electron microscopy and image analysis

2D crystals of reconstituted LHCII trimers were grown as described by Wang and Kühlbrandt (1991) except that digalactosyldiacyl glyceride (DGDG) was added to the crystallization mixture at a ratio of ~2 μg lipid per 1 μg chlorophyll.

Specimens of negatively stained 2D crystals were prepared as described by Kühlbrandt (1984). Electron micrographs were recorded, selected by optical diffraction and digitized by standard techniques (Amos *et al.*, 1982). A projection map was calculated from data of the best one of these images which is complete to ~30 Å resolution.

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